Supplementary Material–*MEP-based biogeochemistry modeling*. Vallino, J.J.

This supplementary material provides addition details on modeling as well as preliminary data on the mesocosms experiments being conducted to test the MEP hypothesis and modeling approach.

Metabolic Half Reactions

The explicit form of the half reactions displayed in Fig. 1 in the main text is given in Table S1.

Table S1. Half reactions used for the methanotroph metabolic network, where $\mathfrak{S}$ is biological structure with a C:N ratio of ρ_S, f_{aC} and f_{aN} are the fraction of assimilated C and N, and dC and dN are detrital C and N, respectively.

Reaction	Structure	Rate
$CH_4 + H_2O \rightarrow CH_2O + 4e^- + 4H^+$	$\mathfrak{S}_1$	r_1
$CH_2O + 2H_2O \rightarrow CO_2 + 4e^- + 4H^+$	$\mathfrak{S}_2$	r_2
$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	$\mathfrak{S}_3$	r_3
$NO_3^- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3H_2O$	$\mathfrak{S}_4$	r_4
$CH_2O + NH_4^+/\rho_S + 0.07e^- \rightarrow \mathfrak{S}$	$\mathfrak{S}_5$	r_5
$\mathfrak{S} \rightarrow f_{aC}\, CH_2O + (1- f_{aC})dC + (f_{aN}\, NH_4^+ + (1-f_{aN})dN)\, /\rho_S + 0.07e^-$	$\mathfrak{S}_6$	r_6
$dC \rightarrow CH_2O$	$\mathfrak{S}_7$	r_7
$dN \rightarrow NH_4^+$	$\mathfrak{S}_7$*	r_8

* Note, $\mathfrak{S}_7$ catalyzes both reactions 7 and 8.

State Equations

For the methanotrophic example, methane in air is sparged into a microcosm of volume V_L at flow rate F_g with gas partial pressures of p^f_{CH4} (0.029 atm), p^f_{O2} (0.202 atm) and p^f_{CO2} (0 atm) (Fig. S1). The microcosm is initially augmented with nutrients (700 μM NO_3^-, 1 μM NH_4^+, and 1 μM CH_2O) and 0.5 μM $\mathfrak{S}_T$, but is otherwise closed except for gas transport. The explicit form of the state equation (Eq. 12) is given by,

$$\frac{dc_{CH4}(t)}{dt} = -r_1(t) + k_L a\left(p_{CH4}/\kappa_{CH4}(T) - c_{CH4}(t)\right)/V_L \tag{S1}$$

$$\frac{dc_{CH2O}(t)}{dt} = r_1(t) - r_2(t) - r_5(t) + f_{aC} r_6(t) + r_7(t) \tag{S2}$$

$$\frac{dc_{NO3}(t)}{dt} = -r_4(t) \tag{S3}$$

$$\frac{dc_{NH4}(t)}{dt} = r_4(t) + \left(f_{aN} r_6(t) - r_5(t)\right)/\rho_S + r_8(t) \tag{S4}$$

$$\frac{dc_{O2}(t)}{dt} = -r_3(t) + k_L a\left(p_{O2}/\kappa_{O2}(T) - c_{O2}(t)\right)/V_L \tag{S5}$$

$$\frac{dc_{CO2}(t)}{dt} = r_2(t) + k_L a\left(p_{CO2}/\kappa_{CO2}(T) - c_{CO2}(t)\right)/V_L \tag{S6}$$

$$\frac{dc_{dC}(t)}{dt} = (1 - f_{aC}) r_6(t) - r_7(t) \tag{S7}$$

$$\frac{dc_{dN}(t)}{dt} = (1 - f_{aN}) r_6(t)/\rho_S - r_8(t) \tag{S8}$$

$$\frac{d\mathfrak{S}_i(t)}{dt} = \sigma_i(t) r_5(t) - \mathfrak{S}_i(t) r_6(t)/\mathfrak{S}_T(t), \quad for\ i = 1, \ldots, 7 \tag{S9}$$

$$\frac{d\zeta_{ATP}(t)}{dt} = 1000/\mathfrak{S}_T(t) \sum_{i=1}^{8} \eta_i(t) r_i(t) \tag{S10}$$

$$\frac{d\zeta_{NADH}(t)}{dt} = 1000/\mathfrak{S}_T(t) \sum_{i=1}^{8} \varepsilon_i r_i(t) \tag{S11}$$

$$\frac{dp_{CH4}(t)}{dt} = F_g\left(p^f_{CH4} - p_{CH4}\right)/V_G + k_L aRT\left(c_{CH4}(t) - p_{CH4}(t)/\kappa_{CH4}(T)\right)/V_G \quad \text{(S12)}$$

$$\frac{dp_{O2}(t)}{dt} = F_g\left(p^f_{O2} - p_{O2}\right)/V_G + k_L aRT\left(c_{O2}(t) - p_{O2}(t)/\kappa_{O2}(T)\right)/V_G \quad \text{(S13)}$$

$$\frac{dp_{CO2}(t)}{dt} = F_g\left(p^f_{CO2} - p_{CO2}\right)/V_G + k_L aRT\left(c_{CO2}(t) - p_{CO2}(t)/\kappa_{CO2}(T)\right)/V_G \quad \text{(S14)}$$

where $\kappa_i(T)$ is the Henry's law constant for gas i, k_L is the liquid-side mass transfer coefficient, a is the air-gas interface area, and V_G is the reactor gas volume.

Experimental Data

Four identical 18 L microcosms (MCs) consisting of a defined mineral salts medium (700 μM NO_3^-, 70 μM PO_4^{3-}, plus salts and trace elements, pH 6.8) were each inoculated with 1 L of whole water collected from a cedar swamp located in Woods Hole, MA on 7 Oct 2005, and reinoculated on day 231. The microcosms (Fig. S1) have been operating continuously for 47 months and are sparged at 20 mL min^{-1} (STP) with a gas mixture of 2.9% methane in air. The microcosms are gently mixed with a cell culture impeller (10 RMP) and maintained in the dark at 20°C. Other than for occasional sampling for chemical analysis (Fig. S2) and associated medium replacement, the reactors are closed except for gas transport, which is continuously monitored for CH_4, CO_2 and O_2 composition (Fig. S3).

Fig. S1. Methanotrophic microcosm (MC2 of 4) sparged with 2.9% methane in air after 47 months of continuous operation. The microcosms are periodically sampled for dissolved and particulate constituents. Note the accumulation of particulate matter at bottom of reactor.

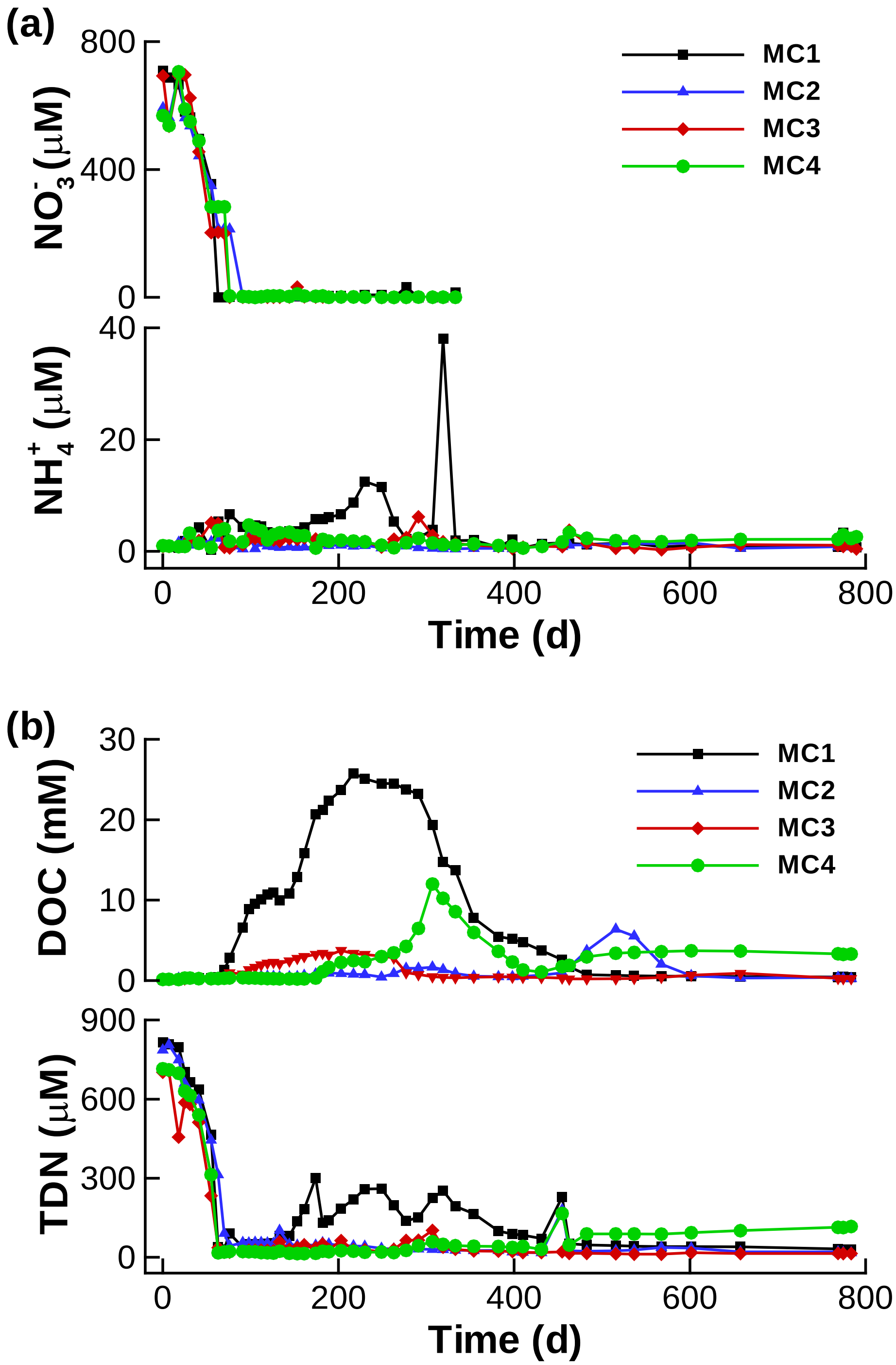

Fig. S2. Preliminary methanotrophic microcosm data for four replicates illustrating changes in (a) nitrate and ammonium concentrations and (b) dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). Note, DOC is plotted as mM. Dissolved organic nitrogen (DON) is obtained by subtracting NO_3^- and NH_4^+ concentrations from TDN concentration. Medium was added on days 63 (1 L), 132 (2 L), and 454 (4.65 L) to replace volume of samples removed for analyses.

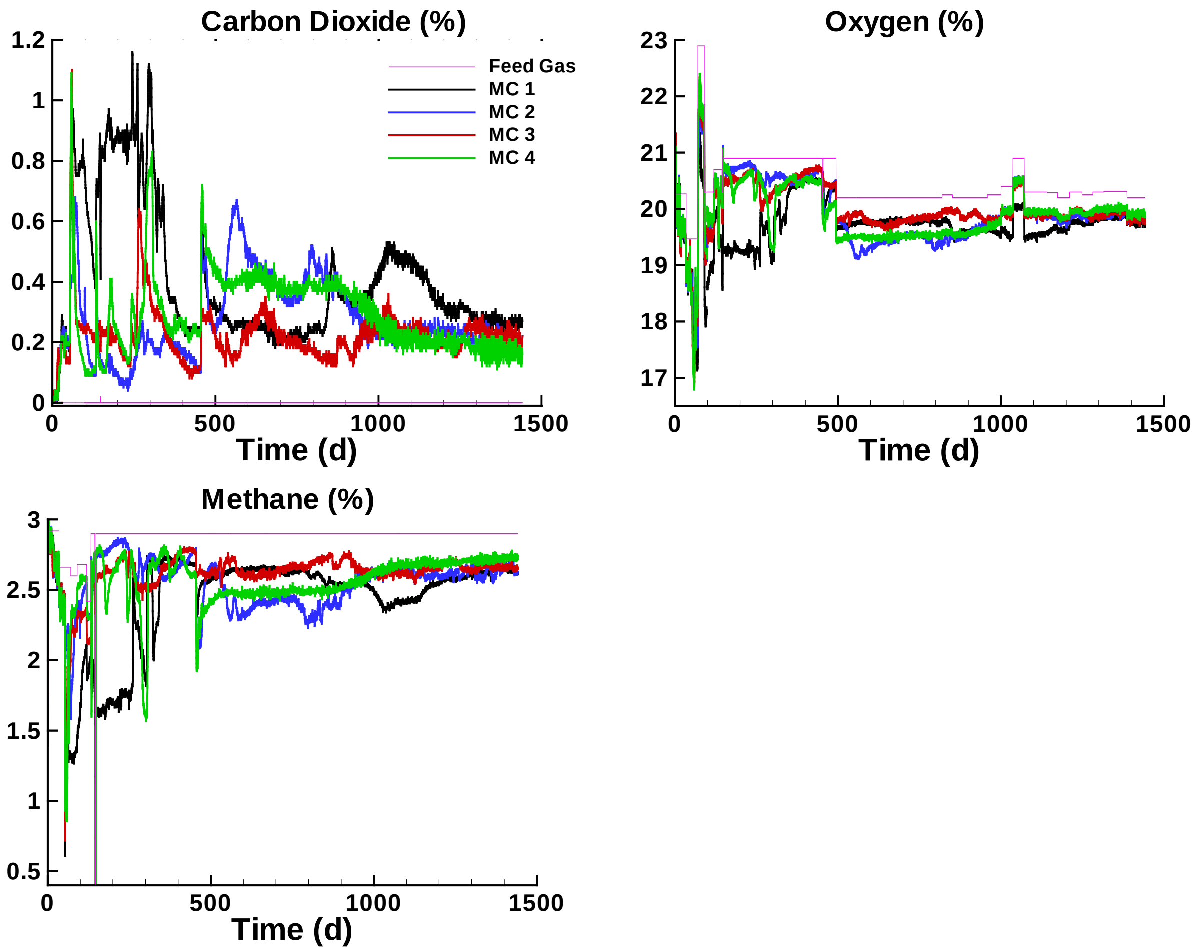

Fig S3. Gas concentrations (%) of CO_2, O_2 and CH_4 entering (purple line) and exiting each of the four replicate microcosms. All microcosms were reinoculated with whole water samples on day 231 because of likely extinction events associated with initial inoculum. Following reinoculation, microcosm dynamics stabilized, but did respond to 4.65 L of medium added on day 454.